Expression of Human Group II PLA₂ in Transgenic Mice Results in Epidermal Hyperplasia in the Absence of Inflammatory Infiltrate

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Abstract

Group II PLA₂ has been implicated in inflammatory processes in both man and other animals and has been shown to be involved in inflammatory conditions, such as arthritis and sepsis. Transgenic mice expressing the human group II PLA₂ gene have been generated using a 6.2-kb genomic fragment. These mice express the group II PLA₂ gene abundantly in liver, lung, kidney, and skin, and have serum PLA₂ activity levels approximately eightfold higher than nontransgenic littermates. The group II PLA₂ transgenic mice reported here exhibit epidermal and adnexal hyperplasia, hyperkeratosis, and almost total alopecia. The chronic epidermal hyperplasia and hyperkeratosis seen in these mice is similar to that seen in a variety of dermatopathies, including psoriasis. However, unlike what is seen with these dermatopathies, no significant inflammatory-cell influx was observed in the skin of these animals, or in any other tissue examined. These mice provide an important tool for examining group II PLA₂ expression, and for determining the role of group II PLA₂ in normal and disease physiology. They serve as an in vivo model for identifying inhibitors of group II PLA₂ activity and gene expression. (*J. Clin. Invest.* 1996. 97:2233–2241.) Key words: transgenic mice • group II PLA₂ • epidermal hyperplasia • hyperkeratosis • inflammation

Introduction

Phospholipases A₂ (PLA₂) are enzymes that catalyze the hydrolysis of the *sn*-2 fatty acyl ester bond of phospholipids to yield a free fatty acid, very often arachidonic acid, and a lysophospholipid (1, 2). PLA₂s can be found in membrane-associated and soluble forms in almost all cell types, where they are believed to play an important role in the normal biosynthesis and turnover of membrane phospholipids (3, 4), cellular signaling (5), and protection of membranes from peroxidation damage (6). In addition, PLA₂s have also been associated with the pathogenesis of numerous clinical inflammatory processes (7, 8).

The rate limiting step in the generation of arachidonic acid derived products in most cell types is controlled by the rate of release of arachidonic acid from phospholipids (9, 10), underscoring the importance of PLA₂ in the generation of these mediators. The oxidation products of arachidonic acid and other polyunsaturated fatty acids play key roles as mediators of inflammation and hypersensitivity. Arachidonic acid is converted by the cyclo-oxygenase pathway to produce a variety of prostaglandins and thromboxanes (11, 12). Specific thromboxanes and prostaglandins have unique effects on blood vessels, smooth muscles, platelets, and other cells resulting in alterations in vessel size, microvascular permeability and platelet aggregation that can influence the course of inflammatory episodes. Arachidonic acid can also be converted by the lipoxygenase pathway to generate hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (13, 14). HETEs and leukotrienes also have the capacity to alter microvascular and smooth muscle function and to initiate and modify leukocyte activities (13, 14). Lysophospholipids, another product of PLA₂ enzymatic activity, can be processed to produce platelet activating factor (PAF) (15).

There are several forms of PLA₂ that can be recognized on the basis of their primary structures (16–20). Group I and group II PLA₂s have been the most extensively characterized. Group I PLA₂ is a family of 14 kD proteins in which the pancreatic digestive enzyme is a major member. Group II PLA₂, also a group of 14-kD proteins, has been found in many different tissues and cell types, such as platelets (21–23), neutrophils (24), vascular smooth muscle cells (25), spleen (26), liver (27), placenta (28), cartilage (29), prostate epithelial cells (30), lacrimal gland cells (31), and the Paneth cells of the intestinal mucosa (32, 33). Both low molecular weight PLA₂ enzymes are characterized by a requirement for millimolar concentrations of calcium, and are secreted enzymes (19). Recently, an 85-kD PLA₂ has been cloned (34) and characterized. This enzyme is present in the cytosolic fraction of many cell types and requires submicromolar concentrations of calcium for activation (35, 36).

Local and systemic levels of PLA₂s are elevated in numerous inflammatory conditions and often correlate with the severity and longevity of the condition. High levels of PLA₂ activity have been demonstrated in the serum of patients with acute pancreatitis (37), in synovial fluids and serum of patients with inflammatory arthritis (38–40), in serum of patients with gram-negative septic shock (41, 42), in serum of patients with adult respiratory distress syndrome (ARDS) (42) and in lesion-free epidermis of patients with pustular psoriasis (43, 44). Recently, group II PLA₂ has been identified as the phospholipase responsible for the elevated PLA₂ activity in patients with acute pancreatitis (45), rheumatoid arthritis, and sepsis and septic shock (46). Furthermore, intraarticular injection of purified group II PLA₂ at physiological concentrations has been shown to cause inflammatory and proliferative changes in synovial structures of both rabbit (47) and rat joints (48). Therefore, it is reasonable to expect that therapeutic agents designed

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to control the level of group II PLA$_2$ activity may have utility in the treatment of these and other inflammatory disorders.

To investigate the role of group II PLA$_2$ in pathological conditions and to provide an animal model to examine the expression of the group II PLA$_2$ gene and the inhibition of the group II PLA$_2$ enzyme, we have generated transgenic mice expressing the human group II PLA$_2$ gene. These mice express the group II PLA$_2$ gene abundantly in liver, lung, kidney and skin and exhibit epidermal and adnexal hyperplasia, hyperkeratosis, and almost total alopecia. These mice provide a valuable model to investigate the role of group II PLA$_2$ in normal and disease physiology as well as an in vivo model to identify and characterize inhibitors of group II PLA$_2$.

Methods

Production of transgenic mice. A human genomic library, prepared by partial digestion of placental DNA with Sau3A and cloned into the EMBL-3 vector (Clontech Laboratories, Inc., Palo Alto, CA), was screened with the human group II PLA$_2$ cDNA (40). Five different clones were isolated which hybridized with the human group II PLA$_2$ probe. By partial sequencing, PCR, and restriction mapping, clone 43A1 was shown to contain the entire PLA$_2$ coding region in addition to 5′ and 3′ flanking sequence. A 6.2-kb HindIII fragment (23) containing 1.6 kb of the 5′ flanking sequence and 0.35 kb of the 3′ flanking sequence was isolated from clone 43A1 and subcloned into the pGEM-3 Z vector (Promega, Madison, WI).

The 6.2-kb HindIII restriction fragment containing the human group II PLA$_2$ gene was microinjected into B6SJLF2 hybrid one cell embryos, which were then transferred to pseudopregnant ICR recipients and developed to term using standard methods (49). Transgenic founders were identified by Southern analysis using a ^{32}P-labeled 800-bp EcoRI fragment isolated from a human group II PLA$_2$ cDNA (40). For Southern analysis, genomic DNA from potential founders was isolated, and 10 μg was digested with BamHI, and electrophoresed on a 0.7% agarose gel. The genomic DNA from transgenic founder mice contained a 6.2-kb BamHI fragment that hybridized to the 800-bp EcoRI fragment. The transgenic founders were bred to C57BL/6J mice to produce G1 animals.

Northern blot analysis. RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction method (50) using an RNA STAT-60 kit (TEL-TEST "B," Inc., Friendswood, Texas). 10 μg of total RNA was subjected to electrophoresis on a 1% agarose-formaldehyde gel and blotted onto Biotrans (ICN Biomedicals, Inc., Costa Mesa, CA). These membranes were hybridized with the 800-bp human group II PLA$_2$ cDNA EcoRI fragment.

PLA$_2$ activity assay. PLA$_2$ activity was assayed according to Davidson et al. (51). Serum samples to be assayed were diluted 1:4 in 100 mM Tris, pH 7.5, 1 mM CaCl$_2$. On ice, 1 μl of these dilutions was added to a 100 μl reaction mix containing 100 mM Tris, pH 7.5, 1 mM CaCl$_2$, and 50,000 cpm ^{3}H-arachidonic acid–labeled *E. coli* membranes (Dupont/NEN, Wilmington, DE). This mixture was incubated at 37°C for 30 min. After stopping the reaction with 50 μl 2N HCl, 50 μl of fatty acid-free bovine serum albumin (20 mg/ml in PBS) was added and the mixture briefly vortexed. After centrifugation in an Eppendorf microfuge at 14,000 *g* for 5 min, the radioactivity released into the supernatant fluid was determined. All of the serum PLA$_2$ activity levels from the transgenic mice were in the linear range of the assay.

Western blot analysis. 1 μl of each serum sample to be assayed was added to 4 μl PBS and 5 μl of 2× sample running buffer (1× concentration: 62.5mM Tris-Cl, pH 6.8, 10% glycerol, 2% SDS, 0.0025% bromphenol blue). These dilutions were heated to 95°C for 2 min and electrophoresed on a 1mm, 14% Tris-Glycine gel (Novex, San Diego, CA) at 125 V. The proteins were electroblotted from the gel to nitrocellulose (0.45 μm pore size, Novex, San Diego, CA) at 30 V for 1 h. The membrane was incubated in PBST (phosphate buffered saline with 0.05% Tween-20) with 5% nonfat dry milk (Carnation Co., Inc. Los Angeles, CA) for 45 min at room temperature and then rinsed twice with PBST. The membrane was then incubated with the primary antibody (monoclonal antibody 4A1-2D6; Boehringer Mannheim, Mannheim, Germany) at 2 μg/ml in PBST with 5% nonfat dry milk for 1.5 h at room temperature, rinsed twice with PBST and washed 3 times with PBST for 5 min each. Goat anti–mouse IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was then incubated with the membrane at a 1:4000 dilution in PBST with 5% nonfat dry milk at room temperature for 1.5 h. After rinsing the membrane twice in PBST and washing three times with PBST for 5 min each, the immunoreactive proteins were detected using the ECL (enhanced chemiluminescence) system (Amersham Corp., Arlington Heights, IL).

Histology and immunohistochemistry. Tissues were fixed in 10% formalin and embedded in paraffin. 5-μm-thick sections were stained with hematoxylin and eosin using standard procedures.

For immunohistochemistry, sections were collected on polylysine-coated slides and reacted with an IgG fraction of a polyclonal rabbit anti–human group II PLA$_2$ antiserum (29). The immunoreaction was then localized (52) using a Vectastain ABC kit (biotinylated secondary antibody, followed by avidin and biotinylated horseradish peroxidase; Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. The slides were counterstained lightly by hematoxylin. As a negative control, the primary antisera was replaced by preimmune rabbit serum.

Skin graft experiments. Ventral trunk skin was grafted onto the thorax of the recipient using a standard procedure (53). Briefly, the donor mouse was sacrificed, shaved, and treated with 70% ethanol to disinfect the skin. The ventral trunk skin was then removed and placed in PBS. The underlying adipose tissue and panniculus carnosus was removed from this donor skin using watchmaker's forceps, and the tissue was cut into pieces of uniform size (∼ 1.5 cm^2). The grafts were kept moistened in PBS. The recipient mice were anesthetized, and the area of the thorax was shaved and disinfected with 70% ethanol. The skin over the thorax was removed, taking care not to remove the panniculus carnosus, the main vascular supply of the skin. The area of skin removed was slightly larger than the size of the donor tissue. The donor skin was placed on the graft bed, and held in place with a sheer Band-Aid® strip (Johnson and Johnson, Inc., Skillman, NJ) saturated with antibiotic (Panolog ointment; J.A. Webster, Inc., Middletown, PA). The recipient mice were closely monitored to insure that the Band-Aids® were not inhibiting their breathing and were re-anesthetized if the Band-Aids® needed to be replaced. After 8 d, the Band-Aids® were removed.

Results

Transgenic mice were generated using a 6.2-kb HindIII genomic fragment of the human group II PLA$_2$ gene (Fig. 1) that includes all 5 exons and 1.6 kb of nucleotides upstream from
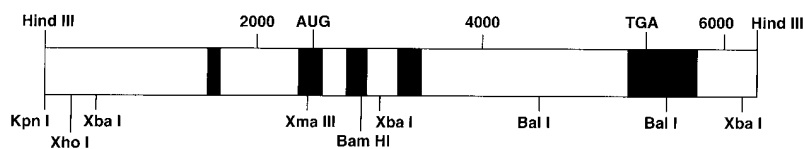

Figure 1. Human group II PLA$_2$ microinjection fragment. A 6.2-kb HindIII fragment containing 1.6 kb of the 5′ flanking sequence and 0.35 kb of the 3′ flanking sequence was used to produce transgenic mice. Solid regions represent exons.
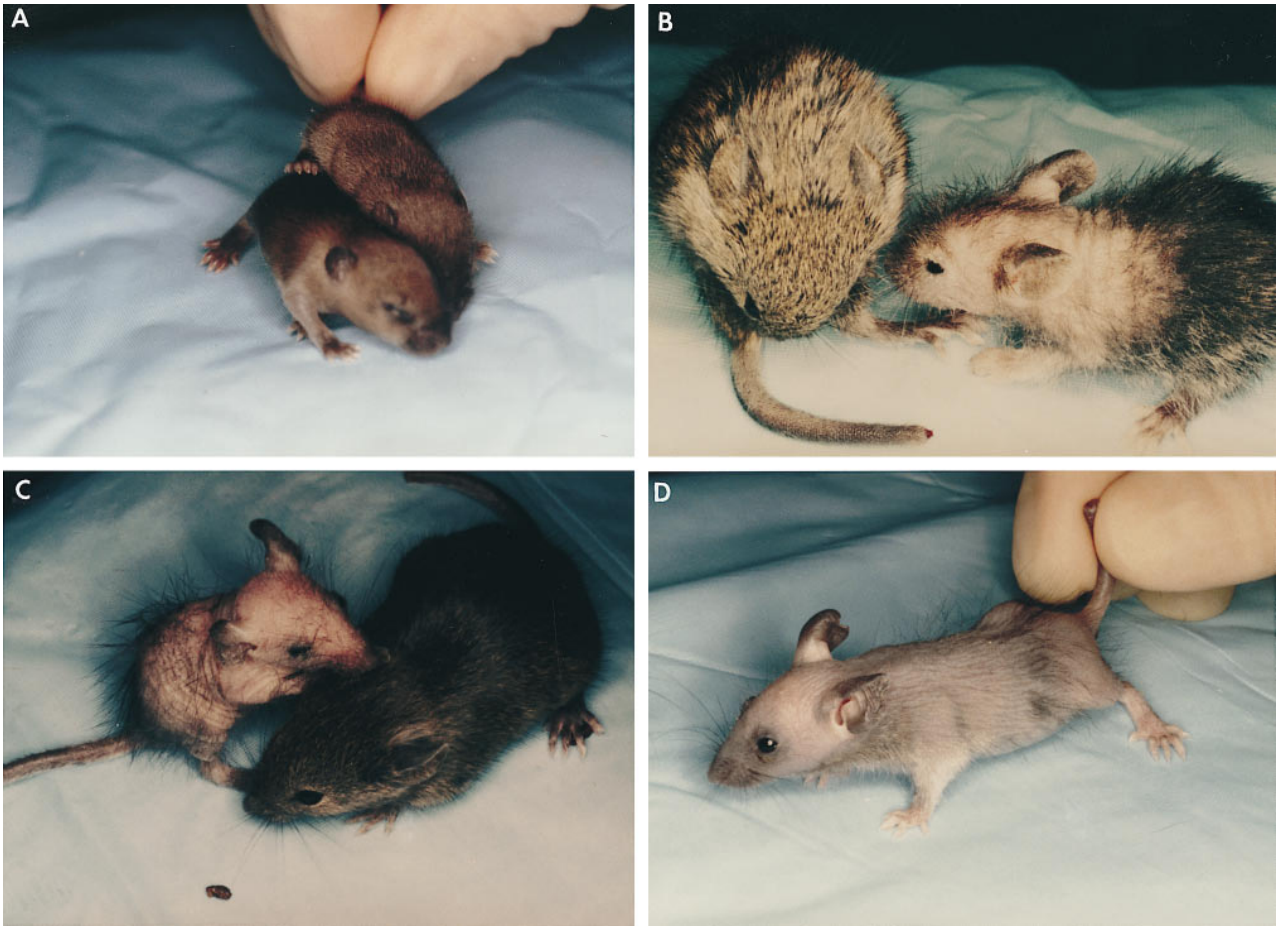
Figure 2. (*A*) 10-d-old founder 854 (*right*) and nontransgenic littermate. (*B*) 17-d-old founder 854 (*right*) and nontransgenic littermate. (*C*) 21-d-old founder 854 (*left*) and nontransgenic littermate. (*D*) 31-d-old founder 854.

the RNA initiation site and 0.35 kb of nucleotides downstream of the polyadenylation signal sequence. 17 founder animals were produced; 10 were observed. By 7–10 d of age, seven of the founder mice (subsequently determined to contain the human group II PLA$_2$ transgene) had coats that were scruffy and sparse compared with their nontransgenic littermates. By 16 d of age the more severely affected founder animals were cachexic and had almost completely lost their body fur. Surprisingly, after 3 wk of age, all of the founder animals had reinitiated hair growth. By 8 wk of age, even the more severely
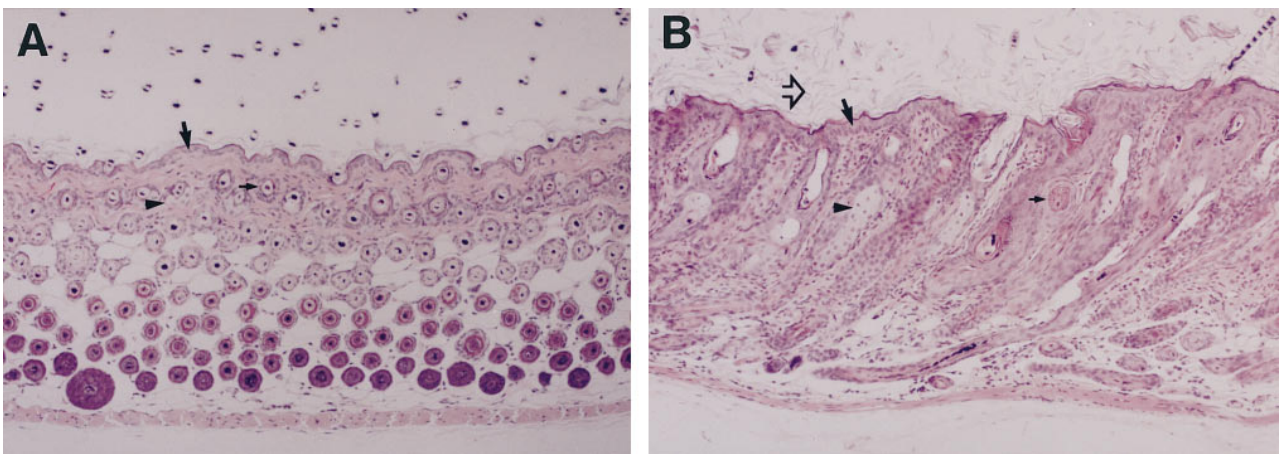

Figure 5. Hematoxylin and eosin stained sections of skin from a 16-d-old nontransgenic (*A*) and a line 703 transgenic (*B*) littermate (×33). Small arrows point to hair follicles, large arrows point to epidermal layer, arrowheads point to sebaceous glands, and the hollow arrow points to hyperkeratosis.
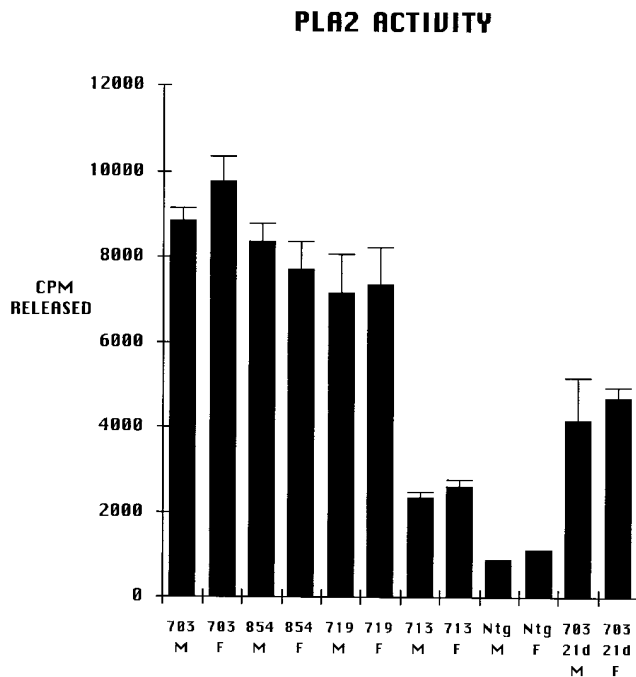
PLA2 ACTIVITY

Figure 3. PLA$_2$ activity assays. PLA$_2$ activity assays were performed as described in Methods. The data is expressed as cpm of ^{3}H-arachidonic acid released from labeled *E. coli* membranes. Each column represents the mean and standard deviation determination of serum samples from three individual adult male (*M*) or female (*F*) mice or 21-d-old mice (*21d*). Differences in PLA$_2$ activity between transgenic groups and nontransgenic (*Ntg*) controls were evaluated using Student's *t* test. Differences were considered significant if $P < 0.05$. The PLA$_2$ activity from all of the transgenic groups were significantly different when compared with the nontransgenic controls.
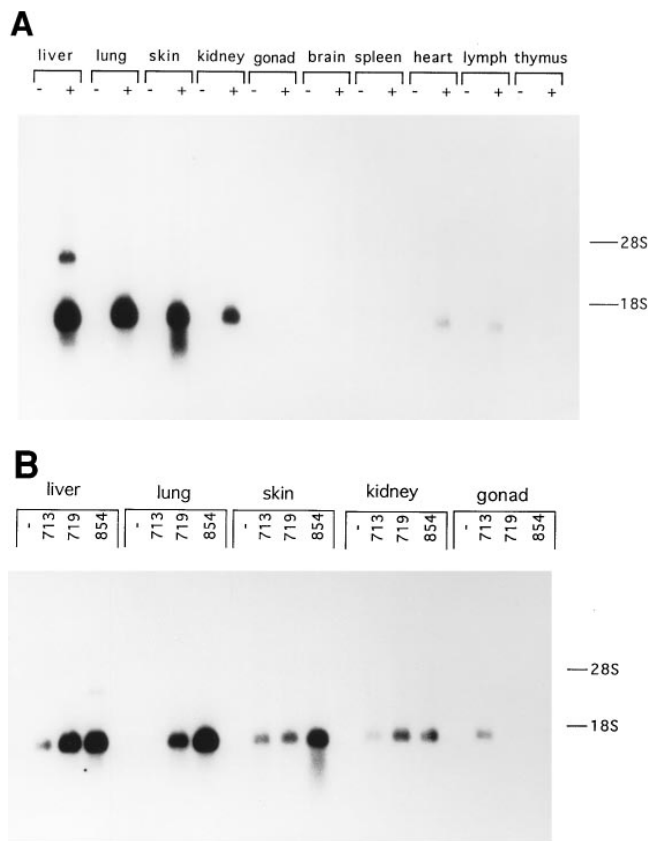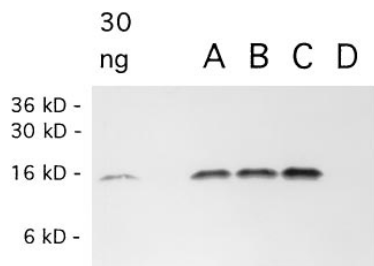

Figure 6. (*A*) Northern blot analysis of RNA isolated from a 21-d-old hemizygous line 854 animal and a nontransgenic littermate. (*B*) Northern blot analysis of RNA isolated from 21-d-old animals from lines 854 (male), 719 (male), 713 (female), and a 21-d-old nontransgenic animal (female).

wasted animals recovered to normal body weights. Adult founders, however, eventually lost most of their coat. Fig. 2 shows a progression of this phenotype in one founder (No. 854).

Founder 854 and three additional founders (Nos. 703, 713, and 719) were bred with C57BL/6 mates to establish transgenic lines. As did the respective founder mice, G1 transgenic mice from each of these lines looked normal during the first 7 d of age. However, as they aged, they all could be distinguished from their nontransgenic littermates because their coats were scruffy and sparse. At ∼ 16 d of age, transgenic mice from the most severely affected lines (854 and 703) developed severe alopecia and were noticeably runted, roughly 25% smaller by weight, compared with their sex-matched litter-

Figure 4. Western blot analysis of serum from 3 line 703, 21-d-old, transgenic siblings (lanes *A, B,* and *C*) and a nontransgenic littermate (lane *D*) using human group II PLA$_2$ monoclonal antibody 4A1-2D6. 30 ng of recombinant human group II PLA$_2$ was electrophoresed for size comparison.

mates. Transgenic mice were ill-appearing and inactive (data not shown). After 21 d, the affected mice reinitiated fur growth but failed to recover all of their fur. Adult mice from lines 854 and 703 remained virtually furless. Adult mice from line 719 and, to a greater extent, line 713, retained a sparse, patchy pelage. There was no direct correlation between the numbers of transgene copies integrated into the genome and the severity of the alopecia and cachexia, as line 703 and 854 had ∼ 2 and 10 transgene copies integrated into the mouse genome, while the less severely affected lines 719 and 713, both had ∼ 1 transgene copy.

PLA$_2$ activity assays were performed on serum isolated from the different lines of PLA$_2$ transgenic mice to determine if they contained elevated levels of PLA$_2$ activity. The results of this analysis are shown in Fig. 3. There was a significant increase in PLA$_2$ activity in serum from adult transgenic mice from all of the lines as compared with serum from nontransgenic littermates or human serum (data not shown for the latter). Line 703 had the highest serum PLA$_2$ activity, followed in relative order of activity by lines 854, 719, and 713. There was a statistically significant difference between the serum PLA$_2$ activity of adult mice from line 703 and the serum PLA$_2$ activity of mice from lines 719, 713, and 21-d-old line 703 mice. Serum PLA$_2$ activity among the different lines appeared to correlate with the severity of the alopecia. Although the severity of the phenotype (alopecia and wasting) was greatest at about 21 d of
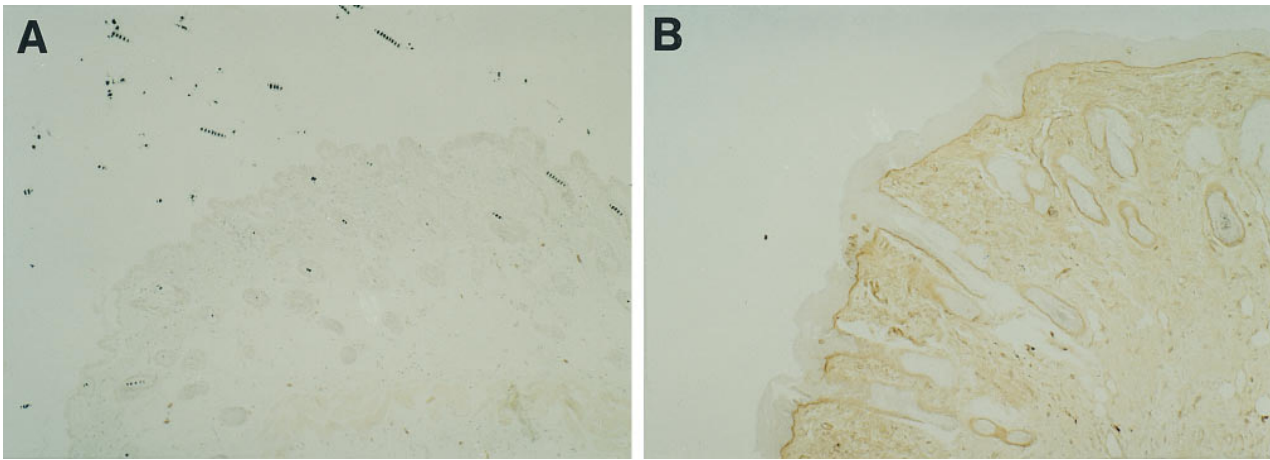
Figure 7. Immunohistochemical analysis of a section of skin from (*A*) a nontransgenic and (*B*) a line 854 transgenic mouse using rabbit anti–human group II PLA$_2$ antiserum (×31).

age in the different lines, adult animals had higher levels of serum PLA$_2$ activity. This data is shown for line 703 in Fig. 3.

To confirm the presence of human group II PLA$_2$ in the serum of these animals, western blot analysis using anti–human group II PLA$_2$ monoclonal antibody 4A1-2D6 was performed. As shown in Fig. 4, an ∼ 14-kD band, which co-migrated with recombinant human group II PLA$_2$, was found in the serum of 21-d-old line 703 mice, but not in a nontransgenic littermate.

The 14-kD band was also present in line 703 transgenic adult mice and the three other transgenic lines (data not shown). The 14-kD band was never observed from the serum of any of the nontransgenic mice.

To investigate the cause of alopecia and wasting observed in the transgenic animals, as well as to look for evidence of inflammatory processes induced by the PLA$_2$ transgene, a histopathological evaluation of these mice was performed. Histopa-
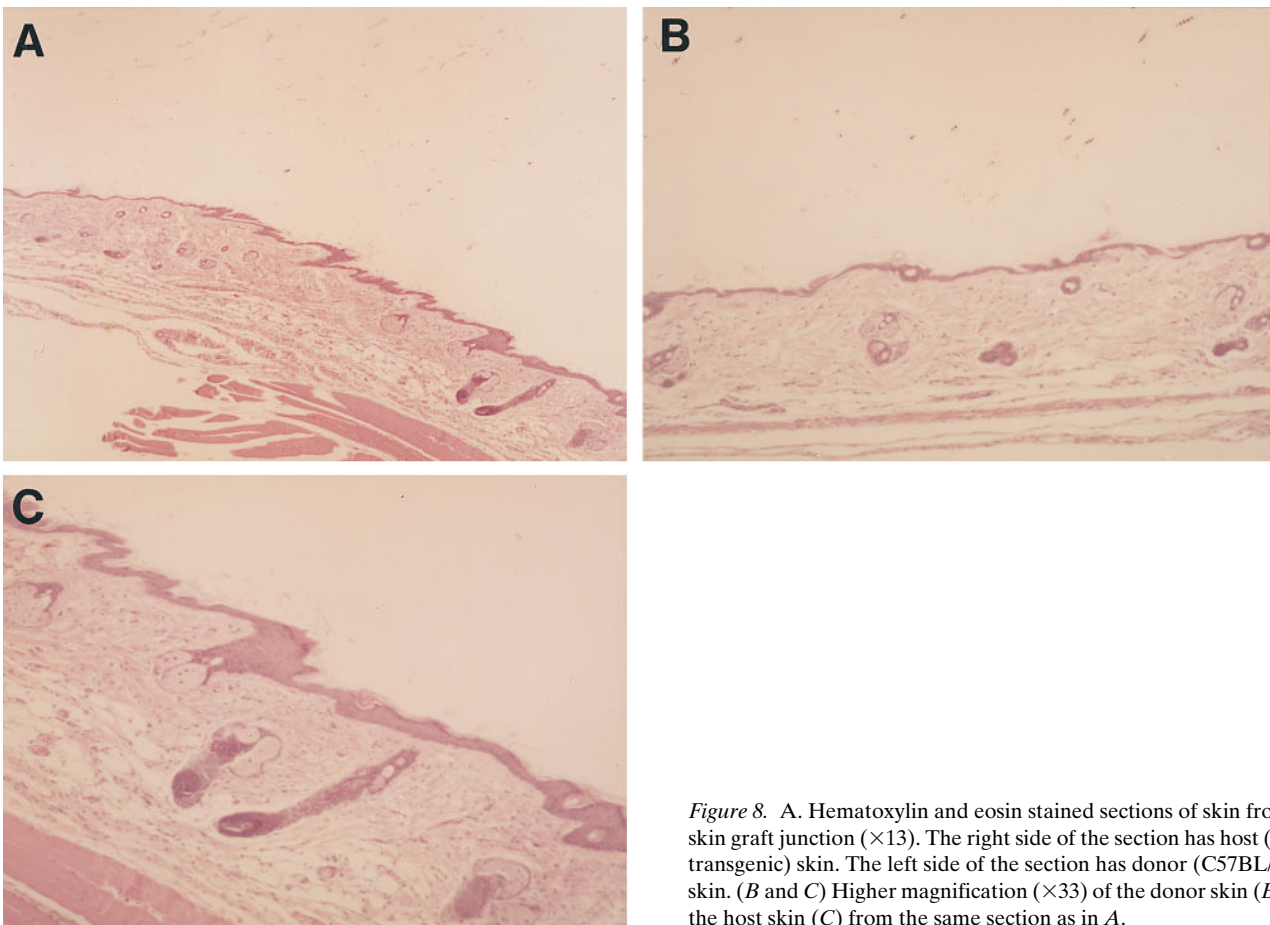

Figure 8. A. Hematoxylin and eosin stained sections of skin from skin graft junction (×13). The right side of the section has host (PLA$_2$ transgenic) skin. The left side of the section has donor (C57BL/6) skin. (*B* and *C*) Higher magnification (×33) of the donor skin (*B*) and the host skin (*C*) from the same section as in *A*.

thology was performed on a variety of organs from 16-d-old animals, when the animals appeared to be most severely affected. Histopathology was also performed on adult organs (data not shown). Hematoxylin and eosin stained sections (5 mm) from each tissue were prepared and analyzed. Skin from 16-d-old and adult transgenic animals had an increase in thickness due to hyperkeratosis, epidermal hyperplasia and adnexal hyperplasia (Fig. 5). Whereas the thickness of the epidermis was 2–3 cells in nontransgenic animals, the epidermal layer in the transgenic animals was 8–10 cells thick. The adnexal hyperplasia was characterized by an increase in the size of the hair follicle with some follicular keratosis or dilation and hyperplasia of the surrounding sebaceous glands. No significant immune cell infiltration was observed in the skin of either the 16-d-old or adult animals. Other organs evaluated (such as bone of the forepaw and hind foot, liver, lung, spleen, pancreas, brain and thymus) did not differ from nontransgenic littermates. No immune cell infiltration was observed in those tissues.

Northern blot analysis was performed to determine the tissue distribution of transgene expression and to compare the relative levels of transgene expression between the different lines of transgenic mice. Total RNA was prepared from a panel of tissues from a 21-d-old line 854 animal. The 800-bp human group II PLA_2 cDNA fragment was used as the probe. An $\sim$ 0.85-kb RNA was detected in almost all tissues examined from the 854 animal. No hybridization to the group II PLA_2 cDNA was observed in tissues from nontransgenic mice, indicating the probe was specific for the human group II PLA_2 RNA or alternatively, expression of murine type II PLA_2 was below the limits of detection. As shown in Fig. 6 A, the most abundant human group II PLA_2 RNA expression was observed in liver, lung, skin, and kidney, with the liver having the highest expression levels. A lower level of human group II PLA_2 RNA expression was observed in the heart and lymph nodes. A similar pattern of expression was observed in an adult line 703 animal; the levels of expression in the liver, skin, kidney, lung, and intestine of this animal were similar to that of an adult line 854 animal (data not shown).

To determine if transgene expression level correlated with the severity of alopecia and wasting observed in these animals, Northern blot analysis was repeated comparing the highest expressing tissues from lines 854, 719, and 713. In Fig. 6 B, Northern blot analysis of RNA from liver, lung, skin, kidney, and reproductive organs was performed on 21-d-old mice from each of these lines. In all three lines, the most abundant expression of human group II PLA_2 RNA was found in the liver. The highest RNA expression in liver was observed in line 854 followed by lines 719 and 713. The expression in liver (the largest source of PLA_2 RNA by weight) correlated with PLA_2 activity in serum. RNA levels in the skin of these animals appeared to correlate with the severity of alopecia (determined by gross observation). The highest RNA levels in skin were observed in line 854, whereas RNA levels in skin observed in lines 719 and 713 were much lower.

To confirm the presence of human group II PLA_2 protein in skin of the transgenic mice, immunohistochemical analysis was performed with human specific antibody to group II PLA_2. As shown in Fig. 7, expression was demonstrated in the dermal layer of the skin. In contrast, staining in the epidermis was limited to weak immunoreactions in a few basal cells. There was no staining observed in the skin of a nontransgenic animal.

Skin graft experiments were performed to determine whether the high circulating level of PLA_2 in the serum, or the expression of PLA_2 within the skin of the transgenic mice, was sufficient to cause the phenotypic abnormalities detected in the skin. Skin from C57BL/6J females was grafted onto line 703 PLA_2 transgenic females (mixed C57BL/6, SJL background). Skin from C57BL/6J females was also grafted onto B6SJLF1 nontransgenic mice as a control. Three months after the grafts were performed, both PLA_2 transgenic animals and nontransgenic animals receiving C57BL/6J skin had hair on the grafted skin (data not shown). Animals were sacrificed at 3 mo after graft and histological analyses were performed. The results (Fig. 8), in the case where human group II PLA_2 transgenic animals were graft recipients, indicated that the donor skin was normal as compared to the host skin (from the PLA_2 animal), which was thickened due to hyperkeratosis and epidermal hyperplasia. Both the donor skin and the host skin were normal in the nontransgenic recipients. This experiment suggests that human group II PLA_2 expression in the skin, and not the elevated serum levels of human group II PLA_2, was responsible for the phenotypic abnormalities detected in the skin of these transgenic mice. The reverse experiment, to determine whether an abnormal skin condition could be reversed in the absence of high levels of serum PLA_2, was not possible because attempts to graft skin from transgenic mice to B6SJLF1 nontransgenic mice or to other (or the same) transgenic animals were unsuccessful. This was most likely due to the abnormal structure of the donor dermis.

Discussion

Transgenic mice expressing human group II PLA_2 were produced to gain insight into the role of group II PLA_2 in inflammatory conditions. Surprisingly, these transgenic mice, which had serum levels of PLA_2 activity significantly higher than that of nontransgenic littermates (8–10-fold higher in lines 703 and 854), did not develop any overt inflammatory conditions, but rather developed a disorder of the skin resulting in alopecia, epidermal hyperplasia, adnexal hyperplasia, and hyperkeratosis. These animals also developed a wasting syndrome starting at $\sim$ 10 d of age, but recovered to normal body weights and activity by $\sim$ 8 wk of age. Despite these conditions, the mice reached adulthood, were fertile, and produced viable offspring. While the severity of these conditions varied between different transgenic lines, the developmental onset and level of severity of these conditions were very similar among the transgenic mice of the same line. Interestingly, transgenic rats expressing the human group II PLA_2 gene had a similar phenotype—no overt inflammatory conditions, but rather a disorder of the skin characterized by alopecia, epidermal hyperplasia, and adnexal hyperplasia, demonstrating that this phenotype is not species specific (unpublished observation).

The transgene fragment used to produce these mice was a 6.2-kb HindIII fragment containing the entire human group II PLA_2 gene. The transcribed region of this gene consists of five exons and four introns spanning 4.2 kb. The 6.2-kb HindIII fragment also contains 1.6 kb upstream and 0.35 kb downstream of the PLA_2 gene. A number of transcriptional response element consensus sequences have been identified within the 1.6 kb upstream region including response elements for IL-6 (54), interferon, hepatocyte NF-3 (55), AP1 (56), AP2 (56), C/EBP (56), and CRE (56). A CCAAT box and TATA

box sequence are also present immediately upstream of the proposed RNA start site. An 0.85-kb human group II PLA_2 RNA was expressed from this transgene fragment in a number of tissues. The tissues containing the highest levels of this RNA were liver, lung, skin, kidney, and intestine (not shown), which was consistent in all four transgenic lines examined. Expression of the human group II PLA_2 RNA was also readily detectable in heart and lymph nodes. The expression in the liver and intestine is consistent with what has been seen in humans. In humans, group II PLA_2 has been detected in hepatocytes both in vivo (unpublished observations) and in vitro (54) and the Paneth cells of the intestinal mucosa (33). Since the expression of genomic transgene fragments often reflect their normal expression patterns, these mice may provide a tool to examine PLA_2 gene expression in vivo.

Serum PLA_2 activity was elevated in all four group II PLA_2 transgenic mouse lines characterized. Mice from the highest expressing lines had serum PLA_2 activity levels 8–10-fold greater than nontransgenic littermates. Recently, it was determined that C57BL/6 mice, as well as several other inbred strains of mice, have a nonfunctional group II PLA_2 allele (57, 58). Our transgenic mice were produced in B6SJLF2 embryos. The mice used in our experiments had been backcrossed an average of three generations to C57BL/6 mice. We have compared the serum activity seen in our transgenic and nontransgenic mice with that of nontransgenic C3H/He and BALB/c mice. Both C3H/He and BALB/c mice have been shown to possess a functional group II PLA_2 allele (58). Therefore, this experiment served to compare the level of PLA_2 activity in our mice with mice containing a completely functional endogenous group II PLA_2 allele. There were similar levels of serum PLA_2 activity in our nontransgenic mice (on a C57BL/6 background) and in both C3H/He and BALB/c mice (data not shown). Therefore, the 8 to 10 fold difference in serum PLA_2 activity between our group II PLA_2 transgenic mice and their nontransgenic littermates was not an overestimate due to the absence of a functional endogenous group II PLA_2 allele. In addition, these results suggest that little of the detectable serum PLA_2 activity in both C3H/He and BALB/c mice is due to group II PLA_2.

Among the four group II PLA_2 transgenic mouse lines characterized, the level of transgene expression, as determined by northern analysis, directly correlated with the severity of the alopecia and the serum PLA_2 activity observed in these animals. There was no correlation between the number of copies of the group II PLA_2 transgene fragment and the level of human group II PLA_2 expression or severity of the phenotype.

Although the serum PLA_2 activity was determined to be higher in adult mice than 21-d-old mice, the cachexia was only evident in nonadult animals. In fact, the human group II PLA_2 transgenic mice lagged behind their nontransgenic littermates in terms of body weight only until ~ 8 wk of age. After this time, the weights of the human group II PLA_2 mice were similar to their nontransgenic littermates. This suggests that expression of group II PLA_2 can have a deleterious effect on the mouse as it matures toward adulthood.

The histopathological analysis of adult and 16-d-old PLA_2 transgenic mice revealed a disorder of the skin consisting of hyperkeratosis, epidermal hyperplasia and adnexal hyperplasia. Chronic, epidermal hyperplasia, and hyperkeratosis in these mice is similar to that seen in a variety of dermatopathies, including psoriasis. However, in contrast to what is seen in psoriasis, and in contrast to reports in the literature indicating that intradermal injection of soluble PLA_2 could trigger inflammation in the skin (59), no significant inflammatory cell-influx in the skin was observed in these animals. The ability of PLA_2 to cause epidermal and adnexal hyperplasia is consistent with reports that TGF-α can stimulate epidermal hyperplasia through the EGF receptor in both tissue culture systems and in transgenic mice (60–62). More recently, EGF receptor signalling has been shown to have an important role in both hair follicle and skin development (63, 64). PLA_2 activity and the subsequent release of arachidonic acid is thought to be an important downstream effect of EGF receptor activation in this pathway. Although evidence suggests the specific PLA_2 protein involved in EGF receptor activation is probably the 85-kD cytosolic protein (65), it is possible that group II PLA_2, when overexpressed in the skin, can trigger a similar response. Alternatively, it is possible that, in this case, group II PLA_2 is interacting with a specific membrane receptor in the dermis or epidermis, directly or indirectly triggering the epidermal cells to proliferate. A 180-kD membrane receptor for group I and group II PLA_2s has recently been identified and cloned (66). Group I PLA_2 has been shown to promote proliferation of Swiss 3T3 cells upon binding to this receptor (67).

Although the mechanism by which the group II PLA_2 triggered the abnormal skin phenotype is not yet known, skin graft experiments were performed to determine the relative capacity of the high circulating levels of group II PLA_2 in the serum and the group II PLA_2 expression in the skin to promote the epidermal hyperplasia. Skin grafts from nontransgenic mice onto PLA_2 transgenic hosts supported hair growth and maintained a normal histology for up to three months. These experiments demonstrated that the circulating levels of PLA_2 activity we observed in these transgenic mice were not capable of causing the epidermal hyperplasia and alopecia, and suggested that expression in the skin of these animals is required to elicit this abnormal skin phenotype. Alternatively, the abnormal skin phenotype may be a developmental phenomenon, with only skin from a young animal (1–3 wk old) being sensitive to high levels of group II PLA_2.

We have not detected any inflammatory cell influx in the skin of 16-d-old, 21-d-old, or adult PLA_2 transgenic mice. Interestingly, inflammatory cell influx can be induced in the skin of mouse ears by topical application of arachidonic acid. In this model of skin inflammation, there is a significant influx of inflammatory cells within days following daily applications (1 mg/ear) of arachidonic acid (68). While continued administration of arachidonic acid results in epidermal hyperplasia, the inflammatory cell influx is less evident after two weeks. Recently, we determined by histopathological analysis that younger animals (ages 7, 10, and 12 d) also lack an influx of inflammatory cells in the skin (data not shown). It is possible, however, that human group II PLA_2 actually did elicit an inflammatory cell influx at an earlier age.

Curiously, there were no abnormalities found in any tissues other than skin by histopathological analysis. The absence of inflammation in the joints of these PLA_2 transgenic mice is at odds with several reports in the literature documenting both the increased levels of group II PLA_2 in serum and synovial fluid isolated from patients with rheumatoid arthritis and the ability of PLA_2 to trigger the arthritic pathologies in animal models (39, 40, 47, 48). Several factors may account for the lack of joint inflammation. While we have documented high

levels of serum PLA$_2$ activity, we have not yet determined the levels of PLA$_2$ activity in the synovial fluid of these transgenic animals. We are currently in the process of determining the cell type specificity of expression of the transgene in joint tissues. Preliminary immunohistochemical analysis of tissue from the joints of these transgenic animals suggests that chondrocytes do indeed express the transgene. However, the level of transgene expression can not be determined from this analysis. The levels of group II PLA$_2$ have been estimated to be between 50 and 500 ng/ml in the synovial fluid of patients with rheumatoid arthritis (47), and inflammation in the joint is dose dependent when group II PLA$_2$ is injected into rabbit joints (47). It is possible that a threshold level of expression needed to induce pathology is not attained in these mice. It is also possible that the transgenic mice will develop joint inflammation as they age. However, we have not detected any evidence of arthritis upon gross observation of animals up to one year of age. Another alternative is that the transgenic animals have developed a compensatory mechanism to attenuate the potential damage caused by high levels of group II PLA$_2$.

In conclusion, the group II PLA$_2$ transgenic mice described here provide a valuable in vivo model to study the role of group II PLA$_2$ in normal and disease physiology. In addition, these mice may provide a useful tool for identifying inhibitors of PLA$_2$ protein activity as well as group II PLA$_2$ gene expression.

Acknowledgments

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